Colony PCR yarrowia

General protocol:
Replate a colony on fresh plate, grow overnight, perform quick and dirty genomic extraction from colonies.

Use TAQ polymerase

If keeping tubes on **ice** – mastermix is ok with TAQ polymerase

Component	**20 µl reaction**
10X Standard *Taq* Reaction Buffer	2 µl
10 mM dNTPs	0.2 µl
10 µM Forward Primer	1 µl
10 µM Reverse Primer	1 µl
Template DNA (genomic extraction)	1 µL
Taq DNA Polymerase	0.1 µl
Nuclease-free water	to 20 µl

STEP	TEMP	TIME
Initial Denaturation	95°C	3 minutes
30 Cycles	95°C 45-68°C 68°C	15-30 seconds 15-60 seconds 1 minute/kb
Final Extension	68°C	5 minutes
Hold	4-10°C	

4.1

cPCR yar F
cPCR yar R
Anneal at 50 °C (64 if phusion)
Extension time: 4:10 (enough for 4.1 kb) for Taq

3970 if insert

4.3

cPCR yar F
cPCR yar R
Anneal at 50 °C (64 if phusion)
Extension time: 3:00 (enough for 3 kb)

2776 if insert

Positive control:
Primers: Gln1 F (JMP1047) and Gln R (tXPR2)
Anneal: 48 degrees (61 if phusion)

Expected product: 1408 bp.